



Attorney Docket No. 27097U

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

BAER et al.

Confirmation No.: 5529

Serial No: 10/562,142

Group Art Unit: 1625

Filed: December 23, 2005

Examiner: DESAI, R.J.

For: **PYRROLO-DIHYDROISOQUINOLINE DERIVATIVES AS PDE10 INHIBITORS**

**Declaration Under 37 CFR 1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

1. I, Dr. Jürgen Braunger, declare and say:
  - 2.1 That I am a citizen of the Federal Republic of Germany, residing at Reichenbacherweg 22, 88444 Ummendorf, Germany.
  - 2.2 That I have expert knowledge of the subject matter of the captioned application for U.S. Letters Patent.
  - 2.3 That I have studied biochemistry at the Eberhard-Karls-Universität Tübingen (1987-1993) and prepared my doctoral thesis at the Ruprecht-Karls-Universität Heidelberg (1993-1997).

- 2.4 That in 1997 I joined Byk Gulden Pharmaceuticals (later re-named to ALTANA Pharma AG and now operating under the name Nycomed GmbH) in Konstanz, Germany, as Postdoctoral Scientist where in 1999 I was promoted to the position of a Senior Scientist. In this position I was, among others, responsible for drug target identification, target validation, the identification and optimization of lead structures, and the screening of pharmaceutically active substances.
- 2.5 That in the year 2007 I assumed a position as Associate Director at Boehringer Ingelheim GmbH in Vienna, Austria, where I was heading a research group in Lead Discovery Oncology, and that, effective Oct 01, 2009, I became Project Leader in R&D<sup>Project</sup> Management at Boehringer Ingelheim GmbH in Biberach, Germany, where I'm leading several preclinical projects and, as team member R&D, I'm contributing to several clinical projects.

2. Summary and Traversal of the 35 U.S.C. §103 rejections

- 2.1 I have intensively studied the Office Action dated June 12, 2009, as well as the cited prior art:

- a) Bauser et al., PCT publication WO 03/014116 A1,
- b) Zhang et al., PCT publication WO 03/051877 A1,
- c) Niewohner et al., PCT publication WO 02/48144 A1,
- d) Ferrari et al., GB 1153670,
- e) Lösel et al., US 4694085,
- f) Liebigs Ann. Chem, vol. 9, pp. 1534-1544,
- g) Casagrande, et al. "Synthesis and Pharmacological Evaluation of Some Pyrrolo[2,1- $\alpha$ ]isoquinolines" (1968), and
- h) Anderson et al., "Synthesis and Murine Antineoplastic Activity of Bis[(carbomoyloxy)methyl] Derivatives of Pyrrolo[2,1- $\alpha$ ] isoquinoline", J. Med. Chem., vol. 27, pp. 1321-1325 (1984),

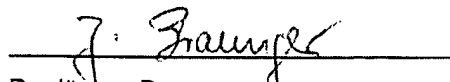
and I am aware that the examiner has rejected claims 1-7, 13 and 16 under 35 U.S.C. § 103(a) as being unpatentable over the disclosures contained in these references, either separately or in combination with each other.

- 2.2 Claim 1 of the present application in the current wording relates to compounds of formula I in which R5 can be alkyl, alkoxycarbonyl, or cyano, but not hydrogen. On the other hand, each of the above cited references teach compounds of formula I in which R5 is hydrogen.

- 2.3 The Examiner alleges in the rejection of claims 1-7, 13 and 16 under 35 U.S.C. § 103(a) as being unpatentable over the cited references that one of ordinary skill in the art would have found it obvious to make the claimed compounds, since compounds which have a close similarity in structure would be expected to have similar properties and hence motivating one to modify them to obtain new compounds.
- 2.4 However, as presented in Appendix A, the compounds as claimed in current Claim 1 of the present application show a totally unexpected biological profile which by no means was suggested or implied by the compounds known from the art. In particular, they show a superior pharmacological profile as PDE10 inhibiting compounds, since they unexpectedly show an increased selectivity for PDE10 over PDE4 when compared to compounds from the art where R5 is hydrogen, and for six of the nine compounds, an increased potency regarding PDE10 inhibition. These findings are completely unexpected in view of the teachings of the prior art which merely show that compounds of formula 1 with R5 being hydrogen are capable of inhibiting PDE10. As such, a person of ordinary skill in the art would not expect that replacing hydrogen in the R5 position by alkyl, alkoxycarbonyl, or cyano would lead to compounds with an increased selectivity for PDE10 over PDE4, and in some respects, an increased potency regarding PDE10 inhibition.
- 2.5 Accordingly, the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 1-7, 13 and 16 under 35 U.S.C. § 103(a).
3. The undersigned Declarant declares further that all statements made herein and in the Appendix of his own knowledge are true and that all statements made on information and belief are believed to be true; further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the application or any patent issuing thereon.

Signed Ummendorf, Germany,

November 07, 2009

  
Dr. Jürgen Braunger

## APPENDIX A

1. The PDE10A is cloned into pCR2.1-Topo (Invitrogen) via PCR from human whole brain cDNA using primers OZ 353 (5'- ACCATGTTGACAGATGAAAAAGTGAAGGC -3') and OZ 317 (5'- TCAATCTTCAGATGCAGCTGCC -3'). The ORF encoding for the PDE10A is cut with EcoRV and BamHI and subcloned into SmaI and Bgl II of the expression vector pBP9 (Clontech). The encoded protein represents the PDE10A1 (GenBank Acc.# AB020593) truncated at its N-terminus at aa 14.

The recombinant baculoviruses are prepared by means of homologous recombination in Sf9 insect cells. The expression plasmids are cotransfected with Bac-N-Blue (Invitrogen) or Baculo-Gold DNA (Pharmingen) using a standard protocol (Pharmingen). Wildtype virus-free recombinant virus supernatants are selected using plaque assay methods. After that, high-titre virus supernatants are prepared by amplifying 3 times. PDE10A1 is expressed in Sf21 cells by infecting  $2 \times 10^6$  cells/ml with an MOI (multiplicity of infection) between 1 and 10 in serum-free SF900 medium (Life Technologies, Paisley, UK). Cells are cultured at 28°C, typically for 48 hours, after which they are pelleted for 5-10 min at 1000 g and 4°C. In spinner flasks, cells are cultured at a rotational speed of 75 rpm. The SF21 insect cells are resuspended, at a concentration of approx.  $1 \times 10^7$  cells/ml, in ice-cold (4°C) homogenization buffer (20 mM Tris, pH 8.2, containing the following additions: 140 mM NaCl, 3.8 mM KCl, 1 mM EGTA, 1 mM MgCl<sub>2</sub>, 10 mM β-mercaptoethanol, 2 mM benzamidine, 0.4 mM Pefabloc, 10 μM leupeptin, 10 μM pepstatin A, 5 μM trypsin inhibitor) and disrupted by ultrasonication on ice. The homogenate is then centrifuged for 10 min at 1000 g (4 °C) and the supernatant is stored at - 80 °C until subsequent use (see below). The protein content is determined by the Bradford method (BioRad, Munich) using BSA as the standard.

The PDE10A activity is inhibited by said compounds in a modified SPA (scintillation proximity assay) test, supplied by Amersham Pharmacia Biotech (see procedural instructions "Phosphodiesterase [3H]cAMP SPA enzyme assay, code TRKQ 7090"), carried out in 96-well microtitre plates (MTPs). The test volume was 100 μl and contained 20 mM Tris buffer (pH 7.4), 0.1 mg of BSA (bovine serum albumin)/ml, 5 mM Mg<sup>2+</sup>, 0.5 μM cAMP (including about 50,000 cpm of [3H]cAMP), 1 μl of the respective substance dilution in DMSO and sufficient recombinant PDE10A1 (1000×g supernatant, see above) to ensure that 15-20% of cAMP was converted under said experimental conditions. After a preincubation of 5 min at 37°C, the reaction is started by adding a substrate (cAMP) and the assays are incubated for a further 15 min; after that, they are stopped by adding SPA beads (50 μl). In

accordance with the manufacturer's instructions, the SPA beads have previously been resuspended in water and diluted 1:3 (v/v) and added to IBMX (3 mM). After the beads have been sedimented (> 30 min), the MTPs are analyzed in commercially available measuring appliances and the corresponding IC<sub>50</sub> values of the compounds for the inhibition of PDE10A activity are determined from concentration-effect curves by means of non-linear regression.

2. The PDE4B2 (GB no. M97515) was a gift of Prof. M. Conti (Stanford University, USA). It was amplified from the original plasmid (pCMV5) via PCR with primers Rb9 (5'-GCCAGCGTGCAAATAATGAAGG -3') and Rb10 (5'- AGAGGGGGGATTATGTATCCAC -3') and cloned into the pCR-Bac vector.

The recombinant baculovirus was prepared by means of homologous recombination in SF9 insect cells. The expression plasmids were cotransfected with Baculo-Gold DNA (Pharmin-gen, Hamburg) using a standard protocol (Pharmin-gen, Hamburg). Wt virus-free recombinant virus supernatants were selected using plaque assay methods. After that, high-titre virus supernatants were prepared by amplifying 3 times. PDE4B2 was expressed in SF21 cells by infecting  $2 \times 10^6$  cells/ml with an MOI (multiplicity of infection) between 1 and 10 in the serum-free medium Insect Express Sf9-S2 (PAA, Pasching, Austria). The cells were cultured at 28°C for 48 – 72 hours, after which they were pelleted for 5-10 min at 1000xg and 4°C.

The SF21 insect cells were resuspended, at a concentration of approx.  $10^7$  cells/ml, in ice-cold (4°C) homogenization buffer (20 mM Tris, pH 8.2, containing the following additions: 140 mM NaCl, 3.8 mM KCl, 1 mM EGTA, 1 mM MgCl<sub>2</sub>, 10 mM β-mercaptoethanol, 2 mM benzamidine, 0.4 mM Pefablock, 10 μM leupeptin, 10 μM pepstatin A, 5 μM trypsin inhibitor) and disrupted by ultrasonication. The homogenate was then centrifuged for 10 min at 1000xg and the supernatant was stored at –80°C until subsequent use (see below). The protein content was determined by the Bradford method (BioRad, Munich) using BSA as the standard.

PDE4B2 activity was inhibited by the compounds claimed in the present application in a modified SPA (scintillation proximity assay) test, supplied by Amersham Biosciences (see procedural instructions "phosphodiesterase [3H]cAMP SPA enzyme assay, code TRKQ 7090"), carried out in 96-well microtitre plates (MTP's). The test volume is 100 μl and contains 20 mM Tris buffer (pH 7.4), 0.1 mg /ml of BSA, 5 mM Mg<sup>2+</sup>, 0.5 μM cAMP (including about 50,000 cpm of [3H]cAMP), 1 μl of the respective substance dilution in DMSO and sufficient recombinant PDE (1000xg supernatant, see above) to ensure that 10-20% of the cAMP is converted under the said experimental conditions. The final concentration of DMSO in the

assays (1 % v/v) does not substantially affect the activity of the PDE investigated. After a preincubation of 5 min at 37°C, the reaction is started by adding the substrate (cAMP) and the assays are incubated for a further 15 min; after that, they are stopped by adding SPA beads (50 µl). In accordance with the manufacturer's instructions, the SPA beads had previously been resuspended in water, but were then diluted 1:3 (v/v) in water; the diluted solution also contains 3 mM IBMX to ensure a complete PDE activity stop. After the beads have been sedimented (> 30 min), the MTP's are analyzed in commercially available luminescence detection devices. The corresponding IC<sub>50</sub> values of the compounds for the inhibition of PDE4B2 activity are determined from the concentration-effect curves by means of non-linear regression.

3. The PDE4D3 (GB no. U50159) was a gift of Prof. Marco Conti (Stanford University, USA). The ORF (GB no. U50159) was cut from the original pCMV5 vector with the restriction enzymes EcoRI and XbaI and subcloned in the expression vector pBP9. This construct was normally used for further experiments. In addition, we also obtained the PDE4D3 (GB no. L20970) from Prof. Marco Conti (Stanford University, USA). It was amplified with oligos OZ 197 (5'- CTGTTACGTGTCAGGAGAACGATC -3') and OZ 90 (5'- GAACATGATGCACGTGAAT -3'), cloned in pCR2.1-Topo to give the plasmid PZ 155 and subcloned with EcoRI in pBP9.

The recombinant baculovirus was prepared by means of homologous recombination in SF9 insect cells. The expression plasmids were cotransfected with Baculo-Gold DNA (Pharmin-gen, Hamburg) using a standard protocol (Pharmin-gen, Hamburg). Wt virus-free recombinant virus supernatants were selected using plaque assay methods. After that, high-titre virus supernatants were prepared by amplifying 3 times. PDE4D3 is expressed in SF21 cells by infecting  $2 \times 10^6$  cells/ml with an MOI (multiplicity of infection) between 1 and 10 in the serum-free medium Insect Express Sf9-S2 (PAA, Pasching, Austria). The cells were cultured at 28°C for 48 – 72 hours, after which they were pelleted for 5-10 min at 1000xg and 4°C.

The SF21 insect cells were resuspended, at a concentration of approx.  $10^7$  cells/ml, in ice-cold (4°C) homogenization buffer (20 mM Tris, pH 8.2, containing the following additions: 140 mM NaCl, 3.8 mM KCl, 1 mM EGTA, 1 mM MgCl<sub>2</sub>, 10 mM β-mercaptoethanol, 2 mM benzamidine, 0.4 mM Pefablock, 10 µM leupeptin, 10 µM pepstatin A, 5 µM trypsin inhibitor) and disrupted by ultrasonication. The homogenate was then centrifuged for 10 min at 1000xg and the supernatant was stored at -80°C until subsequent use (see below). The protein content was determined by the Bradford method (BioRad, Munich) using BSA as the standard.

PDE4D3 activity was inhibited by the compounds claimed in the present application in a modified SPA (scintillation proximity assay) test, supplied by Amersham Biosciences (see procedural instructions "phosphodiesterase [3H]cAMP SPA enzyme assay, code TRKQ 7090"), carried out in 96-well microtitre plates (MTP's). The test volume is 100  $\mu$ l and contains 20 mM Tris buffer (pH 7.4), 0.1 mg /ml of BSA, 5 mM  $Mg^{2+}$ , 0.5  $\mu$ M cAMP (including about 50,000 cpm of [3H]cAMP), 1  $\mu$ l of the respective substance dilution in DMSO and sufficient recombinant PDE (1000 $\times$ g supernatant, see above) to ensure that 10-20% of the cAMP is converted under the said experimental conditions. The final concentration of DMSO in the assays (1 % v/v) does not substantially affect the activity of the PDE investigated. After a preincubation of 5 min at 37°C, the reaction is started by adding the substrate (cAMP) and the assays are incubated for a further 15 min; after that, they are stopped by adding SPA beads (50  $\mu$ l). In accordance with the manufacturer's instructions, the SPA beads had previously been resuspended in water, but were then diluted 1:3 (v/v) in water; the diluted solution also contains 3 mM IBMX to ensure a complete PDE activity stop. After the beads have been sedimented (> 30 min), the MTP's are analyzed in commercially available luminescence detection devices. The corresponding  $IC_{50}$  values of the compounds for the inhibition of PDE4D3 activity are determined from the concentration-effect curves by means of non-linear regression.

4. Table 1 shows pairs of compounds, one compound that falls within the scope of the presently pending claims having an R5 substituent being other than hydrogen and the other compound according to the prior art with R5 being hydrogen. Activities both for PDE10 and for PDE4 were determined following the protocols given above. From these data, given as pIC50 values in the same fields as the compounds' structures, the gains in potency of PDE10 inhibition and in selectivity for PDE10 over PDE4 were calculated and given as non-logarithmic values in the two columns on the right hand side. From these data it becomes obvious that the compounds claimed in the present application show improved pharmacological properties when compared to the compounds known from the art.

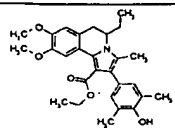
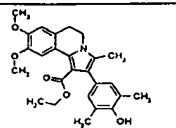
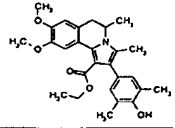
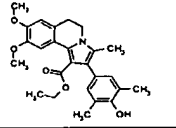
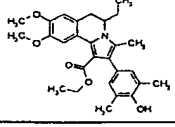
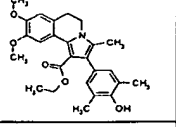
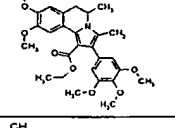
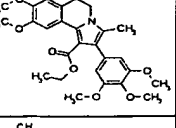
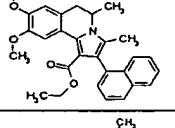
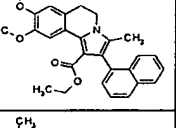
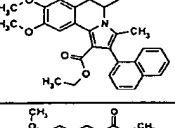
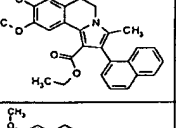
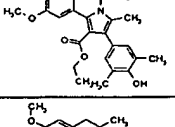
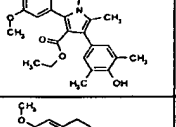
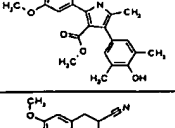
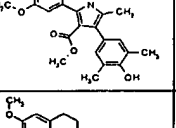
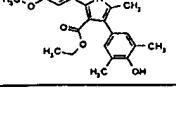
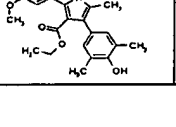
R5 other than H	pIC50(PDE10)	pIC50(PDE4b2)	pIC50(PDE4d3)	R5 = H	pIC50(PDE10)	pIC50(PDE4b2)	pIC50(PDE4d3)	gain in potency of PDE10 inhibition	gain in selectivity for PDE10 over PDE4	gain in potency of PDE10 inhibition (non-logarithmic)	gain in selectivity for PDE10 over PDE4 (non-logarithmic)
	7.4	4.9			7.5	6.4		-0.1	1.4	0.8	25.1
	8.2		5.6		7.5	6.4		0.7	1.5	5.0	31.6
	7.4	4.9			7.5	6.4		-0.1	1.4	0.8	25.1
	7.3	6			6.6	5.8		0.7	0.5	5.0	3.2
	7	4.5			6.3	4.6		0.7	0.8	5.0	6.3
	7	5.2			6.3	4.6		0.7	0.1	5.0	1.3
	7	5.2			7.5	6.4		-0.5	0.7	0.3	5.0
	8	5.8			7.2	5.7		0.8	0.7	6.3	5.0
	8.5	5.6			7.5	6.4		1	1.8	10.0	63.1

Table 1